# Exhibit 1

# Insertion of DNA sequences into the human chromosomal β-globin locus by homologous recombination

Oliver Smithies*, Ronald G. Gregg*, Sallie S. Boggs†, Michael A. Koralewski* & Raju S. Kucherlapati‡

* Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706, USA
† School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, USA
‡ Center for Genetics, University of Illinois College of Medicine, Chicago, Illinois 60612, USA

*A 'rescuable' plasmid containing globin gene sequences allowing recombination with homologous chromosomal sequences has enabled us to produce, score and clone mammalian cells with the plasmid integrated into the human β-globin locus. The planned modification was achieved in about one per thousand transformed cells whether or not the target gene was expressed.*

VARIOUS methods are available for introducing exogenous DNA into mammalian cells[1-5], where it can be stably incorporated into the genome[6,7]. The sites of incorporation have not, however, been under control. Results that could be interpreted as signifying recombination between incoming DNA and a homologous chromosomal gene have been reported by Goodenow *et al.*[8], who transfected mouse L cells with truncated *H-2* genes and observed the expression of full-length H-2 antigens. They did not test this interpretation. Yoshie *et al.*[9] have made similar observations in experiments with truncated *HLA* genes; limited DNA sequence analysis showed that homologous recombination with known *HLA* genes could not account for their data.

The series of experiments reported here shows that homologous recombination between exogenous DNA and a chromosomal gene does occur in cultured mammalian cells and can allow the stable insertion of defined DNA sequences into the human β-globin locus in a predictable fashion. Although the frequency of success ($\sim 10^{-3}$ of the transformed cells) is at present modest, the data show unequivocally that the planned modification of a specific human gene is possible *in vivo*.

We chose the human β-globin locus as a target for gene modification for three reasons: (1) the desirability of eventually being able to correct harmful mutations at this locus in patients with thalassaemia and sickle-cell anaemia; (2) mammalian bone marrow stem cells are likely to become available within the foreseeable future in quantities and purity sufficient to allow tests of the targetted modification with stem cells; and (3) the availability of cloned and sequenced DNA covering much of the locus[10,11].

The rationale used to direct the incoming DNA to the β-globin locus is derived from experiments in yeast by Hinnen *et al.*[12] showing that recombination between incoming DNA sequences and homologous sequences within the genome can direct the exogenous DNA to the desired locus. A refinement, based on work in yeast by Orr-Weaver *et al.*[13], is to cut the incoming DNA within the region of homology using a suitable restriction enzyme. In a previous study[14], we showed that the provision of such recombinogenic ends greatly increases homologous recombination between plasmids in mammalian somatic cells. This suggests that cutting within the region of homology may also significantly increase the frequency of directed gene modification in mammalian cells.

## Detecting target gene modification

Figure 1 shows the planned modification to the human β-globin locus, that is, the integration by homologous recombination of a single copy of a test plasmid into the locus at a defined position. To achieve this modification, we constructed a special test plasmid, pΔβ117, that could also be used in a sensitive screening

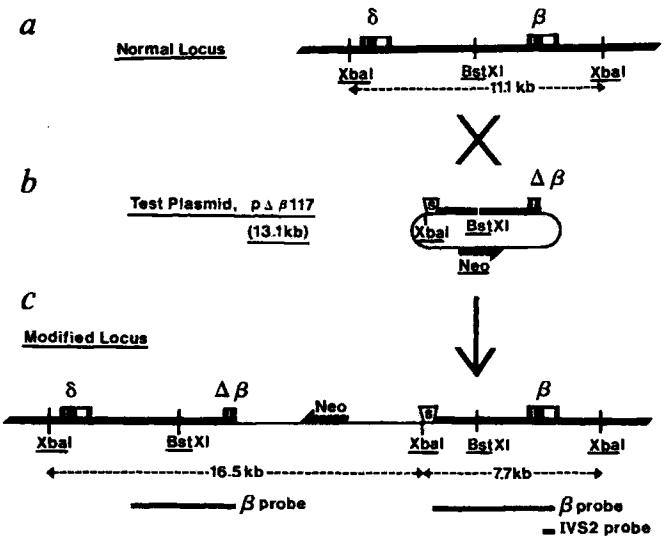


**Fig. 1** Planned modification of the human β-globin locus. *a*, Map of normal human DNA encompassing the adult δ- and β-globin genes, with their coding regions and introns indicated by solid and open bars. The transcriptional sense of these genes is from left to right. All *Xba*I sites are shown. Only one of two *Bst*XI sites in the region is indicated; the other, omitted for clarity, is in the third exon of the β-globin gene. *b*, The 13.1-kb test plasmid, pΔβ117, used as a vehicle to achieve and detect the targetted modification. Human globin locus DNA sequences (4.6 kb) in the test plasmid are indicated by a heavy line that is aligned appropriately with *a*. (The sequences extend from the first *Eco*RI site upstream of the β-globin gene to the *Bam*HI site at the 3' end of the second exon.) The 5' part (Δβ) of the β-globin gene within these sequences includes the first exon and most of the second exon (shown by solid bars) and the first intervening sequence (open bar) but does not include the second intervening sequence, IVS-2. The light line shows sequences largely derived from the plasmid pSV2Neo[16] from which pΔβ117 was constructed. The *neo* gene is indicated by a crosshatched arrow (not to scale) that also shows its transcriptional orientation. The boxed letter S (not to scale) represents the *supF* gene, which encodes a tyrosine tRNA able to suppress some amber mutations in *E. coli*. The *supF* gene was taken from the plasmid pπVX of Seed[17] together with a polylinker that provided the adjacent *Xba*I site. The naturally occurring *Bst*XI site within the globin sequences of pΔβ117 is indicated; this is the only *Bst*XI site within the test plasmid. It was cut as indicated before introducing the plasmid into recipient cells. *c*, Map of the β-globin locus as it should appear after the planned modification by homologous recombination. Horizontal bars at the bottom of *c* show regions in the modified β-globin locus to which the two probes used in the assay will hybridize.

**Table 1** Fragment rescue from cells not expressing (EJ) or expressing (Hu 11) the β-globin gene

| Cells | G418-resistant colonies | Total genomic DNA assayed (μg) | Total phage* | Phage growing on C-1a | Phage hybridizing to β probe | Phage hybridizing to β and IVS-2 probes† |
|---|---|---|---|---|---|---|
| EJ carcinoma (no G418) | [405]‡ | 100 | $6.3\times10^8$ | 2,520 | 125 | 1 |
| EJ carcinoma (no G418) | [1,530]‡ | 50 | $3.4\times10^8$ | 820 | 24 | 1 |
| Hu 11 hybrid (+G418) | 4,400 | ~20 (5.5–8.5-kb fraction) | $2.4\times10^8$ | 1,420 | 145 | 2 |
| Hu 11 hybrid (+G418) | 4,400 | ~20 (8.5–16.5-kb fraction) | $1.4\times10^8$ | 1,760 | 60 | 0 |

Cells were transformed with pΔβ117 cut with *Bst*XI and grown in the absence or presence of G418, as indicated below DNA was prepared, digested with *Xba*I and assayed for fragment rescue. Nine dishes of human EJ bladder carcinoma cells ($5\times10^5$ per dish) were treated with calcium phosphate precipitates of carrier-free pΔβ117 DNA, linearized by cleavage with *Bst*XI, at either 1 μg (first line) or 8 μg (second line) per dish. After 3–4 days of culture in medium without G418, the cells were collected for DNA preparation and the DNA was digested with *Xba*I. $10^8$ Hu 11 hybrid cells (see text), grown in HAT medium, were suspended in 1 ml 140 mM NaCl, 25 mM HEPES, 0.75 mM $Na_2HPO_4$, *p*H 7.15, in the presence of 25 μg $ml^{-1}$ *Bst*XI-digested pΔβ117 DNA. Electroporation[4,24] was carried out in an ice-cooled 0.5 ml treatment vessel with 1 $cm^2$ platinum end-electrodes. A solid state electronic switch was used to discharge a bank of condensers (effective capacitance 14 μF charged to 1,000 V) through the treatment vessel. HAT medium was added 10 min after the shock and the cell suspension was dispensed into four culture flasks, except for a small amount used for estimating transformation frequencies. Two days later an equal volume of HAT medium containing G418 was added to all cultures to give a final concentration of 400 μg G418 $ml^{-1}$. Subsequent medium changes every three days used HAT medium plus 400 μg G418 $ml^{-1}$. DNA was prepared from the cells grown in each of the four culture flasks (estimated to contain 1,100 G418-resistant colonies per flask), and from these samples a pool of DNA was prepared equivalent to 4,400 colonies. After *Xba*I digestion the pooled DNA was separated by agarose electrophoresis into two fractions: 5.5–8.5 kb and 8.5–16.5 kb. The *Xba*I-digested DNA in all the experiments was ligated to DNA from the double-amber mutant bacteriophage Charon 3AΔXba, made from Charon 3AΔLac[25] by adding an *Xba*I cloning site and deleting 1.8 kb (net) of DNA to increase its cloning capacity to ~11 kb. The ligated DNA was packaged[26] into viable phages using sonic extracts and freeze-thaw lysates prepared from *recA*⁻ strains of *E. coli*. The total number of phages was assayed on *E. coli* strain K802, which suppresses the amber mutations. Phages that rescued the *supF* gene from the genomic DNA or lost their amber mutations during the packaging (see text) were assayed on late logarithmic cells of *E. coli* strain C-1a, which cannot suppress amber mutations. Phage plaques on strain C-1a were blotted twice with nitrocellulose and hybridized to two probes (see Fig. 1): a β probe which will hybridize to the human globin sequences in the test plasmid pΔβ117, and an IVS-2 probe which will hybridize only to phages containing the 3′ part of the β-globin gene that was not in the incoming test plasmid. (The β probe is a 5,548-bp *Eco*RI fragment that includes the 5′ part of the human β-globin gene; the IVS-2 probe is a 917-base pair *Bam*HI/*Eco*RI fragment that spans the β-globin IVS-2 sequence.)

* This total, obtained from titration on *E. coli* K802, includes recombinant and non-recombinant phages, because the Charon 3AΔXba vector phage will grow on K802 with or without an insert.

† These doubly hybridizing phages were examined at the DNA level by electrophoresis and were shown to contain the predicted 7.7-kb *Xba*I fragment.

‡ The cells used for DNA preparation were not exposed to G418. These estimates of G418-resistant cells are calculated from small parallel samples.

assay to detect and measure the frequency of the targetted modification[15]. Because it is a screening method, the assay allows a target gene to be chosen for which no direct selection method is available. It depends on rescuing DNA sequences corresponding to the input DNA from the genome of the recipient cells, together with genomic sequences next to this DNA, and then determining whether the adjacent sequences correspond to the expected region of the target locus.

The test plasmid pΔβ117 incorporates four important elements. The first is 4.6 kilobases (kb) of DNA from the human β-globin locus, including the 5′ part of the adult human β-globin gene but lacking the second intervening sequence (IVS-2) and the remaining 3′ portion of the gene. This DNA provides sequences in the incoming test plasmid that can recombine with homologous target sequences in the β-globin locus of the recipient cell to accomplish the planned modification. Within the 4.6-kb element is a single *Bst*XI site used to cut the incoming plasmid in the region homologous with the target.

The second element is sequences from the plasmid pSV2Neo of Southern and Berg[16] that specify the *neo* gene and associated transcriptional signals. These sequences can confer resistance to G418 (an antibiotic related to neomycin) on mammalian cells. In some experiments, we used G418 to select recipient cells that had incorporated the test plasmid in a configuration in which the *neo* gene was functional.

The third element in pΔβ117 is the *supF* gene, which encodes a tyrosine transfer RNA that suppresses several amber mutations in *Escherichia coli* and its bacteriophages. When this suppressor gene is inserted into a mutant bacteriophage, carrying amber mutations preventing the phage from growing, the amber mutations will be suppressed and the phage will grow. We used a double-amber mutant bacteriophage cloning vector (Charon 3AΔXba[15]) in a variation of a scheme described by Seed[17] to rescue *supF*-containing sequences from the recipient mammalian cell genome. Only those phages that have rescued the *supF* gene will, in principle, grow on a wild-type strain of *E. coli* (C-1a) that lacks amber suppressors. (In practice, phages also grow that have lost both their amber mutations by recombination during packaging of the phage DNA[18], but these phages do not contain any globin sequences and so do not compromise the assay).

The fourth important element in pΔβ117 is a site for the restriction endonuclease *Xba*I located upstream of the *supF* gene relative to the human β-globin sequences. In conjunction with a second *Xba*I site downstream of the adult β-globin gene in the normal human genome, this *Xba*I site generates a novel 7.7-kb *Xba*I fragment of DNA when homologous recombination has accomplished the planned modification (see Fig. 1). The 7.7-kb *Xba*I fragment is unique among the *supF*-containing genomic fragments rescued during the assay because it is the only one that contains both the *supF* gene and the IVS-2 part of the β-globin gene.

Thus, the overall procedure consists of the following stages: (1) introduction of the test plasmid DNA into the recipient cells; (2) in most experiments, selection with G418 of those cells that have incorporated the test plasmid into their genomes; (3) preparation of DNA from pools of these transformed cells; (4) digestion of DNA from the cell pools with the restriction enzyme *Xba*I; (5) ligation of the resulting genomic DNA fragments to DNA from the double-amber mutant vector phage (Charon 3AΔXba), packaging of the ligated DNA into viable phages and plating them on *E. coli* C-1a which lacks any suppressor genes;

**Table 2** Fragment rescue from pools of transformed Hu 11 hybrid cells

| Pool description | Number of colonies | Total genomic DNA assayed (μg) | Total phage* | Phage growing on C-1a | Phage hybridizing to β probe | Phage hybridizing to β and IVS-2 probes |
|---|---|---|---|---|---|---|
| Flask 1 | 1,100† | 40 | $1.3\times10^9$ | 2,650 | 381 | 1 |
| Flask 2 | 1,100† | 20 | $2.6\times10^8$ | 800 | 60 | 1 |
| Flask 3 | 1,100† | 20 | $2.7\times10^8$ | 570 | 61 | 2 |
| Flask 4 | 1,100† | 40 | $9.4\times10^8$ | 1,630 | 265 | 0 |
| Flask 5 | 300‡ | 20 | $2.2\times10^8$ | 150 | 25 | 3 |
| Flask 6 | 100‡ | ~40 | $2.8\times10^8$ | 440 | 41 | 0 |
| Pool 1 | 20‡‖ | 40 | $7.5\times10^7$ | 115 | 36 | 8¶ |
| Pool 2 | 20‡‖ | 20 | $3.3\times10^7$ | 53 | 22 | 0 |
| Pool 3 | 20‡‖ | 20 | $2.2\times10^7$ | 25 | 11 | 0 |

Hu 11 hybrid cells were transformed by electroporation to G418 resistance as in Table 1, except as noted below, and were cultured in flasks or as pools of individual colonies as indicated. DNA was isolated, digested with *Xba*I and assayed as described in Table 1.

* Total includes recombinant and non-recombinant phages.

† Estimated from a small sample.

‡ Pooled from individual G418-resistant colonies obtained following electroporation in the presence of 12.5 μg ml$^{-1}$ pΔβ117 cut with *Bst*XI.

‖ Cells from a frozen sample of flask 5 were thawed, recloned, amplified and pools of 20 individual colonies were prepared; as a consequence of this procedure the colonies in these pools are no longer independent.

¶ Representative phages were examined at the DNA level by electrophoresis, and were shown to contain the predicted 7.7-kb *Xba*I fragment. The *Xba*I fragment from one doubly hybridizing phage was subcloned and shown, by further digests, to have the expected map, including the *supF* gene.

(6) scoring the resulting phages for hybridization to two probes (see Fig. 1). The first probe (the β-probe) identifies phages that have rescued any human globin sequences derived from the incoming test plasmid. The second probe (the IVS-2 probe) is made from the IVS-2 portion of the human β-globin gene that was absent from the incoming test plasmid but will be next to plasmid-derived globin sequences after the planned modification. Doubly hybridizing phages detect and score the occurrence of the modification. They can be purified and further checked by electrophoresis to ensure that they contain the predicted 7.7-kb *Xba*I fragment.

## Preliminary work

In initial experiments[15] we had transformed human EJ bladder carcinoma cells to G418 resistance with various test plasmids of the same general design as and including pΔβ117, but no successful modification of the native β-globin gene was detected in ~3,000 independent G418-resistant colonies sampled. This result was surprising because we were successful when using an incoming plasmid to correct a defective chromosomal plasmid that we had introduced into the genome as an artificial target[15] (see also similar experiments by Smith and Berg[19] and Lin *et al.*[20,21] and related experiments with viral sequences by Shaul *et al.*[22]). A possible explanation for this initial failure is that the combination of G418 selection and bladder carcinoma cells that do not express their β-globin genes is inappropriate. Specifically, any carcinoma cells in which the desired modification has been achieved might not survive G418 selection if the location of the inserted DNA within an inactive chromosomal locus prevents expression of the *neo* gene. Two sets of tests were carried out to avoid this problem. In the first, G418 selection was omitted. In the second, we used recipient cells in which a human adult β-globin gene was being expressed.

## Tests without G418 selection

Human EJ bladder carcinoma cells not expressing their β-globin genes were used as recipients for incoming plasmid DNA in two experiments omitting G418 selection. Details of these experiments and their results are presented in Table 1. One phage from each experiment hybridized to both probes. Each was plaque-purified and its DNA was shown to contain the expected 7.7-kb *Xba*I fragment. Additional digests with *Eco*RI and *Bam*HI confirmed that the *Xba*I fragment had the map expected for a complete β-globin gene[11] including IVS-2. These experiments are therefore strong evidence that the locus has been modified in the predicted fashion in these cells not expressing the β-globin gene, and that the modification can be detected when G418 selection is omitted. Although the experiments were based on the supposition that the *neo* gene cannot be expressed when inserted into an inactive β-globin locus, they do not prove that the supposition is correct.

## Cells expressing a human β-globin gene

The second set of tests required a clonable recipient cell expressing the human adult β-globin gene. A cell line described by Zavodny *et al.*[23] meets these criteria. It was made by fusing hypoxanthine phosphoribosyltransferase-deficient tetraploid mouse erythroleukaemia cells to diploid human fibroblasts bearing an X;11 translocation chromosome and selecting for growth in medium containing hypoxanthine, aminopterin and thymidine (HAT medium). The resulting hybrid cell line, which we term the Hu 11 hybrid, expresses the adult β-globin gene on human chromosome 11 at a measurable level in the absence of induction, and at a high level after induction with dimethyl sulphoxide[23].

Hu 11 hybrid cells were transformed to G418 resistance by pΔβ117 cut with *Bst*XI using electroporation[4] as described in Table 1. A pool of DNA representing 4,400 G418-resistant colonies of transformed cells was prepared and digested with *Xba*I. Agarose gel electrophoresis was used to prepare two size fractions from this DNA. One fraction covered the size range 5.5–8.5 kb and the other the range 8.5–16.5 kb. Using this size-fractionated DNA served two functions: it reduced the total amount of DNA to be packaged and it eliminated a conceivable artefact, namely that the diagnostic 7.7-kb *Xba*I fragment might be formed from the 11-kb *Xba*I fragment that includes the β-globin locus in unmodified recipient cells (see Fig. 1) by homologous recombination during the phage packaging[20] or subsequent steps of the assay.

The results obtained with the two fractions are shown in Table 1. Two doubly hybridizing phage were detected with the 5.5–8.5-kb fraction but none was found in the 8.5–16.5-kb fraction. Because the doubly hybridizing phages were obtained with <8.5 kb DNA, this result excludes the possibility that they were formed from the unmodified 11-kb *Xba*I fragment during the assay. The doubly hybridizing phages were plaque-purified and shown to contain the predicted 7.7-kb *Xba*I fragment. These experiments thus provide strong evidence that the planned modification has been achieved in Hu 11 hybrid cells expressing the β-globin locus and that G418 selection does not kill the

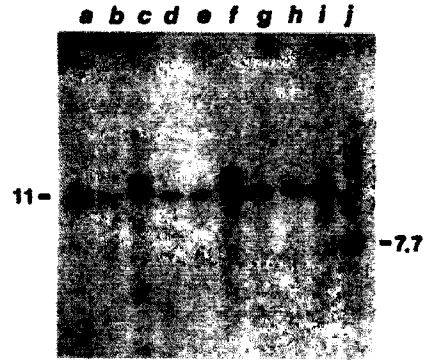


**Fig. 2** Nitrocellulose blot of *Xba*I digests (lanes *a*–*j*) of DNA from 10 G418-resistant colonies (A–J) from pool 1 (see text and Table 2) hybridized to the IVS-2 probe specific for the human β-globin gene. Measured fragment sizes are shown in kb.

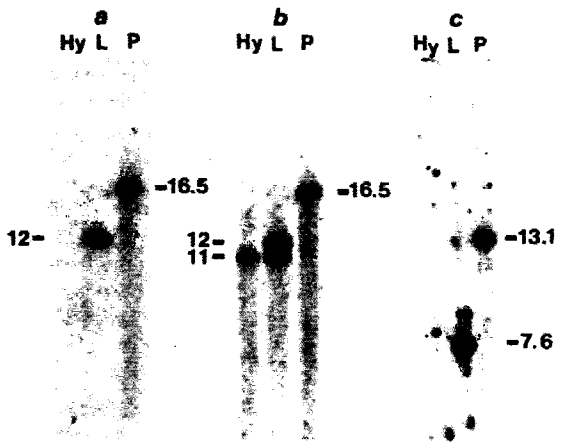


**Fig. 3** Nitrocellulose blots of digests of DNA from two of the G418-resistant colonies from pool 1 (see text and Table 2) and from untreated Hu 11 hybrid cells. The blots were: *a*, *Xba*I digests hybridized to a probe specific for pSV2Neo-derived sequences; *b*, *Xba*I digests hybridized to probes specific for the IVS-2 of the human δ-globin gene and for pSV2Neo sequences; *c*, *Bst*XI digests hybridized to a probe specific for pSV2Neo sequences. Samples are: Hy, untreated Hu 11 hybrid cells; L, the G418-resistant colony L; P, the G418-resistant colony P. Measured fragment sizes are shown in kb.

resulting cells. Accordingly, we used the assay in conjunction with a sib-selection procedure to identify and isolate from the G418-resistant cells a clone containing the modified β-globin locus.

## Clone isolation

The pooled DNA studied in the preceding section was made from four flasks of cells each containing an estimated 1,100 G418-resistant colonies. In similar experiments we prepared two flasks containing pools of 300 or 100 isolated individual colonies, respectively (see Table 2 for data obtained with these six flasks). Three of the 1,100-colony flasks and the 300-colony flask scored positively, whereas the other two flasks were negative. Thus, the presumptive modification is being accomplished about once per 300 to once per 1,100 cells transformed to G418-resistance.

Because the 300-colony flask scored positviely, we thawed a frozen sample of cells from this flask and recloned them. The resulting clones were expanded and samples were taken to create several 20-colony pools. (Note that because these colonies are subclones from one flask, they are no longer independent.) DNA from three of the 20-colony pools was tested; pool 1 scored positively whereas pools 2 and 3 did not (Table 2). Accordingly, we prepared and tested directly DNA samples from each of the 20 individual colonies constituting pool 1.

## Direct test of modification

Figure 1 shows how the planned addition of the 13.1-kb test plasmid to the human β-globin locus will alter the restriction map of the locus. The most obvious predictions are that the diagnostic 7.7-kb *Xba*I fragment should be detectable by the β-globin IVS-2 probe in Southern blots of DNA from the modified cells, and that this fragment should replace an 11-kb *Xba*I fragment present in the DNA of unmodified cells.

Figure 2 shows a Southern blot of *Xba*I digests of DNA from 10 of the 20 colonies comprising pool 1. In one of the colonies (J) the predicted change is seen; a 7.7-kb *Xba*I fragment has replaced the usual 11-kb fragment from the unmodified locus. (The same result was also seen in another colony, P, in the remaining 10 colonies of pool 1.) As no residual 11-kb fragment is present, the modified colony probably has only one copy of the β-globin locus. (Karyotyping of comparably treated Hu 11 hybrid cells showed only one chromosome with the banding pattern of human chromosome 11.) Thus, the fragment rescue assay for the planned modification is validated in that it led us to two not necessarily independent colonies in which the modification is demonstrable by direct tests.

## Fidelity of modification

To determine whether the modification is the sought-for simple one-copy insertion of pΔβ117, digests of genomic DNA from one of the modified colonies (P) were hybridized to several probes (Fig. 3). As controls, DNA was included from untreated Hu 11 hybrid cells and from one of the other colonies (L) in which pΔβ117 was incorporated at a non-globin location.

Figure 3*a* shows *Xba*I digests of the samples hybridized to a probe (pSV2Neo) that will detect most of the non-globin parts of inserted pΔβ117 sequences. The modified colony P gives only one hybridizing band at 16.5 kb. This shows that the map is altered as expected (see Fig. 1) from the insertion of only a single copy of pΔβ117 between the δ- and β-globin genes, and it also shows that no plasmid sequences were incorporated into other (illegitimate) sites in the genome of the modified colony. Colony L also gives a single hybridizing band indicating the incorporation of pΔβ117 sequences into the recipient cell genome at only a single location, but the size of the fragment (12 kb) demonstrates that the incorporation was not at the targetted site. The untreated Hu 11 hybrid cells show no plasmid sequences.

Figure 3*b*, obtained by hybridizing a probe specific for the human δ-globin gene to the blot used for *a* (already hybridized to pSV2Neo) confirms and extends these conclusions. Colony P still shows only the single 16.5-kb band that contains both plasmid sequences and the δ-globin gene. In contrast, colony L now shows two bands, because the plasmid sequences and the δ-globin gene are on different *Xba*I fragments (12 and 11 kb, respectively). The untreated Hu 11 hybrid cells show only the 11-kb fragment from the unmodified locus.

Because *Bst*XI was used to create recombinogenic ends on the incoming plasmid, a *Bst*XI digest of DNA from the modified colony is particularly informative. Accurate insertion of a single copy of the test plasmid without any net degradation should result in two *Bst*XI sites (see Fig. 1) separated by the length of the plasmid (13.1 kb). Figure 3*c* shows that such a 13.1-kb fragment, detected by the plasmid-specific pSV2Neo probe, is indeed present in *Bst*XI digests of DNA from the modified colony P. Colony L, having the plasmid inserted at an illegitimate site, gives a different and unpredictable *Bst*XI fragment at 7.6 kb.

We conclude that the modification observed in the Hu 11 hybrid cells was the sought-for insertion of a single copy of the test plasmid into the β-globin locus. As the modified colonies are G418 resistant, our results also establish that the *neo* gene inserted into an active β-globin locus can be expressed even though its transcriptional orientation is opposite from that of the β-globin gene.

## Conclusions

The experiments reported here establish that the planned modification of a specific human gene can be accomplished in mammalian cells by homologous recombination without detectably affecting other parts of the genome. Our assay data indicate that the modification was achieved whether or not the target gene was active. When it was active we were able to use selection of the inserted sequence to help clone the modified cells. Although many other problems remain to be solved, these results suggest that specific planned modifications of the $\beta$-globin locus in bone marrow stem cells may eventually be possible for the treatment of patients with haemoglobinopathies such as thalassaemia and sickle-cell anaemia. Regardless of whether this long-term goal can be made practical, it is evident that extensions of the principles we have used here to other genes in complex eukaryotes should permit new approaches to many problems in molecular genetics.

This work was supported by grants to O.S. (AM-20120, GM-20069) and to R.S.K. (GM-33943, March of Dimes I-806). We thank Deb Endean and Paula Henthorn for help with p$\Delta\beta$117 and Charon 3A$\Delta$Xba, Natalie Borenstein for tissue culture, Anne Hanneken and Charlie Blystad for assays, Brian Morse and Stacy Ehrlich for DNA preparations, Lisa Becker for freeze-thaw lysates, Art Skoultchi for Hu 11 hybrid cells, Huntington Potter and Bill Sugden for advice on electroporation and Karen Lyons for suggestions concerning our early failutres. This is paper number 2834 from the Laboratory of Genetics, University of Wisconsin-Madison.

Received 1 July; accepted 30 July 1985.

1. Wigler, M. *et al. Cell* **11**, 223-232 (1977).
2. Capecchi, M. R. *Cell* **22**, 479-488 (1980).
3. Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7380-7384 (1980).
4. Neumann, E., Schaefer-Ridder, M., Wang, Y. & Hofschneider, P. H. *EMBO J.* **1**, 841-845 (1982).
5. Miller, A. D., Jolly, D. J., Friedmann, T. & Verma, I. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4709-4713 (1983).
6. Palmiter, R. D. *et al. Nature* **300**, 611-615 (1982).
7. Chada, K. *et al. Nature* **314**, 377-380 (1985).
8. Goodenow, R. S. *et al. Nature* **301**, 388-394 (1983).
9. Yoshie, O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 649-653 (1984).
10. Fritsch, E. F., Lawn, R. M. & Maniatis, T. *Cell* **19**, 959-972 (1980).
11. Poncz, M., Schwartz, E., Ballantine, M. & Surrey, S. *J. biol. Chem.* **258**, 11599-11609 (1983).
12. Hinnen, A., Hicks, J. B. & Fink, G. R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1929-1933 (1978).
13. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6354-6358 (1981).
14. Kucherlapati, R. S., Eves, E. M., Song, K.-Y., Morse, B. S. & Smithies, O. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3153-3157 (1984).
15. Smithies, O., Koralewski, M. A., Song, K.-Y. & Kucherlapati, R. S. *Cold Spring Harb. Symp. quant. Biol.* **49**, 161-170 (1984).
16. Southern, P. J. & Berg, P. *J. molec. appl. Genet.* **1**, 327-341 (1982).
17. Seed, B. *Nucleic Acids Res.* **11**, 2427-2445 (1983).
18. Kataoka, T., Takeda, S.-I. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2666-2670 (1983).
19. Smith, A. J. H. & Berg, P. *Cold Spring Harb. Symp. quant. Biol.* **81**, 171-181 (1984).
20. Lin, F.-L., Sperle, K. K. & Sternberg, N. *Cold Spring Harb. Symp. quant. Biol.* **81**, 139-149 (1984).
21. Lin, F.-L., Sperle, K. & Sternberg, N. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1391-1395 (1985).
22. Shaul, Y., Laub, O., Walker, M. D. & Rutter, W. J. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3781-3784 (1985).
23. Zavodny, P. J., Roginski, R. S. & Skoultchi, A. I. *Globin Gene Expression and Hematopoietic Differentiation* (eds Stamatoyannopoulos, G. & Nienhuis, A. W.) 53-62 (Liss, New York, 1983).
24. Potter, H., Weir, L. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7161-7165 (1984).
25. Blattner, F. R. *et al. Science* **196**, 161-169 (1977).
26. Hohn, B. *Meth. Enzym.* **68**, 299-309 (1979).

# LETTERS TO NATURE

## New upper bound on the flux of cosmic magnetic monopoles

**A. D. Caplin, C. N. Guy, M. Hardiman*, J. G. Park† & J. C. Schouten**

Blackett Laboratory, Imperial College, London SW7 2BZ, UK

**Grand unification theories (GUTs) suggest that massive magnetic monopoles (mass $\sim 10^{16}$ GeV) were created in the very early stages of the formation of the Universe. The report, 3 years ago[1], of a candidate cosmic magnetic monopole therefore stimulated intense experimental and theoretical activity[2,3]. These monopoles should have the magnetic charge $h/e$ predicted by Dirac[4], or perhaps small integer multiples of $h/e$, but zero electric charge. Surviving magnetic monopoles are thought to be moving so slowly, $v/c = 10^{-3}$ or less, that they interact exceedingly weakly with matter. For this reason, conventional particle detectors (whose mechanism is electronic ionization or excitation) are expected to be rather insensitive[5], so that inductive detectors are particularly attractive. We describe here the results of 6 months observation with a large inductive detector. Some putative events have been seen, but none of them seem to have been induced by a monopole. Our total exposure (detector area–time product) is now about 230 times that of Cabrera's first experiment, so that it seems increasingly likely that his original candidate event was spurious.**

A Dirac monopole traversing an isolated closed conducting loop induces a current, by Faraday's law of induction, that generates magnetic flux equal to $h/e$ ($=4.1\times10^{-15}$ Wb), equivalent to $2\Phi_0$, where $\Phi_0$ is the magnetic flux quantum associated with superconductivity. No other particle will induce a net current, and furthermore the size of the current is independent of the trajectory and speed of the monopole. In practice, the detector loop is best made superconducting, so that the induced current is permanent and more easily measurable. Fluctuations in the ambient magnetic field would also cause flux changes in the detector loop, so that good magnetic screening is essential. A convenient and effective way to do this is to surround the detector loop with a superconducting shield; however, the magnetic coupling (mutual inductance) between the loop and the shield complicates matters: the larger the size of the loop relative to the shield, the greater the coupling and the more the magnitude of the induced current in the loop depends on the details of the monopole trajectory.

Cabrera's original detector was a 50-mm diameter loop within a 200-mm diameter superconducting shield, and his detection of a single candidate event after 6 months of observation was at odds with the astrophysical limit[6] on the number flux of cosmic monopoles derived from the persistence of galactic magnetic fields. Cabrera's single candidate monopole, if genuine and if interpreted as a rate, implied a number flux some 6 orders of magnitude greater than this astrophysical upper bound.

Our inductive detector contains three independent superconducting detector loops within a single superconducting shield, 960 mm high and 234 mm in diameter (Fig. 1). The two horizontal loops each have a single turn of diameter 170 mm and a counterwound 10-turn coil of diameter 54 mm to reduce the magnetic coupling to the shield[7]. The 'window-frame' (WF) loop, in contrast, is deliberately strongly coupled to the shield, but it is sensitive to all monopole trajectories intersecting the shield other than those nearly on axis[8]. While this design maximizes the effective detector area, it does sacrifice the valuable identifying feature of a single well-defined signal magnitude from passage of a monopole. There is partial coincidence between the three loops: trajectories that intersect either the top (T) or bottom (B) loops have high probability, $\approx$95%, of giving a significant signal in the WF loop. The probability of coincidence between B and T loops is $\approx$7%. Only about 10% of the tracks giving a signal in the WF loop intercept either the B or T loops. Each of the detectors is fitted with a toroidal calibration coil that can be energized externally, so as to simulate a monopole. Our inductive detector is, we believe, one of the two largest now in operation, being comparable in size with that

* Present address: School of Mathematical and Physical Sciences, University of Sussex, Falmer, Brighton BN1 9QH, Sussex, UK.
† Deceased 22 June 1983.